PATENT
Attorney Docket No. 260449US0XPCT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of	)	
	)	
KOSMATOPOULOS	)	
	)	Group Art Unit: 1643
Serial No.:10/511,273	)	
	)	Examiner: BRISTOL, Lynn Anne
Filed: 06/27/2005	)	
	)	
For: EPHA2 ANTIGEN T EPITOPES		

Declaration pursuant to 37 C.F.R. § 1.132

Hon. Commissioner of Patents
and Trademarks
Washington, D.C. 20231

I, Kostas KOSMATOPOULOS, do hereby declare and state the following

1. That I am Doctor of Medicine (1974, Faculty of Medicine, University of Thessaloniki, Greece), Doctor of Philosophy (1985, University of Thessaloniki, Greece), Docteur d'Etat en Biologie Humaine (1986, University Paris XI, France), Research Director in INSERM (Institut National de Santé et de la Recherche Médicale, France), Head of the laboratory of tumor immunotherapy at the Gustave Roussy Institute (Villejuif, France) and currently Director of R&D in Vaxon Biotech. My *curriculum vitae* and a list of scientific publications, which clearly indicate my expertise in the field of immunotherapy, were attached to my previous Declaration, dated May 12, 2006.

2. I am the inventor of the above-captioned patent application and therefore I am very familiar with the subject application. I have read and understood the Official Action issued by the U.S. Patent and Trademark Office on 10/15/2007. It is my understanding that Claims 1-3 and 7 were rejected under 35 U.S.C. § 103(a), as being unpatentable over Schirle *et al.* (J. Immunol. Methods, 2001) in view of Powell *et al.* (USPN 20070031882) as evidenced by Tatsumi et al. (Cancer Res., 2003) and Parker et al. (J. Immunol., 1994). In rendering this rejection, the Examiner asserts that one skilled in the art at the time the claimed invention was made would have been motivated to produce the claimed immunogenic peptide and would have been assured of reasonable success in doing so based on the combined disclosures of Schirle, Powell, Tatsumi and Parker.

3. In order to address the issue of obviousness based on the above cited references, I would like to point out the following elements.

4. First of all, I would like to point out that Tatsumi disclosure is dated 2003, August whereas the present patent application was filed earlier in 2003, April. So, Tatsumi disclosure should not have been included in the prior art.

5. Concerning the other references, I would like to submit the following scientific elements, which demonstrate that the skilled artisan was not motivated to obtain immunogenic T epitopes from the EphA2 protein by combining Powell, Schirle and Parker documents.

6. Powell *et al.* propose the use of antibodies against EphA2 protein in order to modulate its activity in HIV infected T cells. As disclosed in paragraph [0276], page 41, these antibodies can be prepared by immunizing a suitable subject (*e.g.*, rabbit, goat, mouse or other mammals) using standard techniques. Human EphA2 is xenogeneic "non self" for rabbit, goat and mouse and generation of humoral or cellular immune response against xenogeneic "non self" proteins is very common. Since human EphA2 is an autologous self protein, it will be very unlikely that this protein generates a humoral and cellular immune response in human. Moreover, antibodies generated in animals are directed against B-epitopes recognized by the cognate animal immune system but not by the human immune system. The skilled artisan knows that the presence in a human protein of B-epitopes recognized by the animal immune system does not mean that said protein should have B-epitopes and more improbably T-epitopes recognized by the human immune system.

7. Schirle *et al.* (J. Immunol. Methods 257:1-16, 2001) teache prediction algorithms for proteasomal cleavage. In the introduction section, the authors explain that the discovery in the 1990's of peptide motif for every MHC-molecule was the first step for the development of the reverse immunology approach which the authors describe as the most successful strategy for T cell epitopes identification.
As stated by the authors page 2, right column, last two sentences of the first paragraph, the reverse immunology approach shows a "high failure rate" due namely to identification of peptides not produced by the proteasome. Schirle et *al* introduce prediction algorithms for proteasomal cleavage in addition to reverse immunology in order to obtain more relevant results. Nevertheless, only 2 (second and third peptides of the table) over the 4 peptides presented table 1 page 4 are T epitopes predicted to be produced by the proteasome using PAPROC.

8. Morerover, Shirle *et al* refers to an article by Kessler *et al* (J.Exp.Med. 193:73-88, 2001, already cited in my previous Declaration), which discloses a study on PRAME antigen (page 7, column 1, last paragraph). Only 4 out of the 19 high affinity binders peptides were found to be T epitopes. We have tested these 4 epitopes using PAPROC and FRAGPREDICT algorithms and considered prediction of proteasomal cleavage when both algorithms gave a positive result simultaneously. None of these 4 epitopes was predicted by both algorithms in these conditions.

9. These results on PRAME peptides also remind that high affinity of a peptide for a HLA molecule is not sufficient for this peptide to be an epitope. Peptide must be produced by the proteasome digestion of the protein from which it is derived (see my

previous Declaration dated 12/05/2006). Kessler *et al.* also report that only 21% of high affinity HLA molecule binding peptides were found to be efficiently generated by the proteasome and presented by said HLA molecule. Parker *et al.* disclosure (J. Immunol., 152: 163, 1994), which teaches the BIMAS program to find high HLA affinity peptide, does not provide any information as to how to determine if a high HLA affinity peptide is an epitope.

10. We have also tested our claimed immunogenic peptides (see Claim 2) in PAPROC and FRAGPREDICT algorithms and found that only two of them (SEQ ID NO: 6 and SEQ ID NO:8) were predicted to be epitopes, although these peptides are all naturally produced by the proteasome.

11. Predictive models of proteasome cleavage are also used in one of my publications (Vaccine, 24:2102, 2006, enclosed), which deals with a polypeptide vaccine composed of three different peptides. This work teaches that only one out of six combinations of the 3 peptides described allows for the cleavage of all three peptides and elicits a trispecific immune response. We found that the predictive models, PaProc and Netchop, were unable to predict the real sites of cleavage (page 2105, left column, 3.2) within the polypeptide and failed to identify the optimal polypeptide candidate. This recent publication shows that prediction algorithms for proteasomal cleavage are still irrelevant to identify immunogenic T epitopes.

12. Reading the article by *Parker et al.*, the skilled artisan could apply the BIMAS program to the EphA2 protein only to identify high HLA affinity peptides. But the skilled artisan would not have found any motivation in Schirle *et al.* proteasomal cleavage algorithms, nor in Powell *et al.* B-epitopes, to determine if EphA2 contains T-epitopes. No information is given in Parker *et al* about how to identify a T-epitope produced by proteasome cleavage from a pool of high affinity peptides, and the skilled artisan was not assured of reasonable success by using prediction algorithms for proteasomal cleavage as exposed by Schirle *et al.*

13. As a conclusion, I am sincerely convinced that it would **not** have been obvious for one of ordinary skills in immunology at the time the claimed invention was made, to obtain EphA2 immunogenic T-cell peptides, on the basis of the disclosures of Schirle, Powell and Parker.

14. I further declare that all statements made herein of my knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine and emprisonment, or both under section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of this application or any patent issuing thereon.

Date February 8th, 2008

Kostas KOSMATOPOULOS